Raman Spectroscopy of Wet Blue Bovine Leather**

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Abstract

A comprehensive, multi-instrument Raman spectroscopic study of wet blue bovine leather was performed in order to detect the weak, low frequency, organo-chromium vibrations in chrome tanned leather. Raman scattering spectroscopy is a complementary measurement to infrared absorption spectroscopy. Both types of vibrational spectroscopies theoretically enable the direct measurement of chromium-collagen interactions. Wet blue bovine leather was studied in two forms, slices and powder, by several different instruments (dispersive, Fourier Transform and holographic spectrometer). This paper is an overview of the instrumental and sampling requirements for high quality spectral measurements of this unique sample type. Observed peaks and spectral features are discussed. Implications for protein microstructure and process analysis applications are presented.

Resumen

Un comprensivo estudio multi-instrumental espectroscópico tipo Raman de cuero bovino wet blue fue efectuado para así detectar las delicadas vibraciones, de bajas frecuencias, órgano-crómicas en cuero simplemente curtido al cromo. Espectroscopia de dispersión Raman es una medición complementaria a la espectroscopía por absorción infrarroja. Ambos tipos de vibraciones determinables por espectroscopía teóricamente permitirían directamente la determinación de las interacciones cromo-colágeno. Cuero wet-blue bovino se estudió sobre dos formas, tajadas y pulverizadas, por varios diferentes instrumentos (dispersión, Transformaciones de Fourier y espectrómetro holográfico). Esta obra es una visión extensa de la instrumentación y muestreo requeridos para medidas espectroscópicas de alta calidad sobre este tipo de muestra tan especial. Los picos y las características de los espectros son discutidas. Implicaciones acerca de la microestructura proteínica y los procedimientos analíticos aplicables son presentados.

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INTRODUCTION

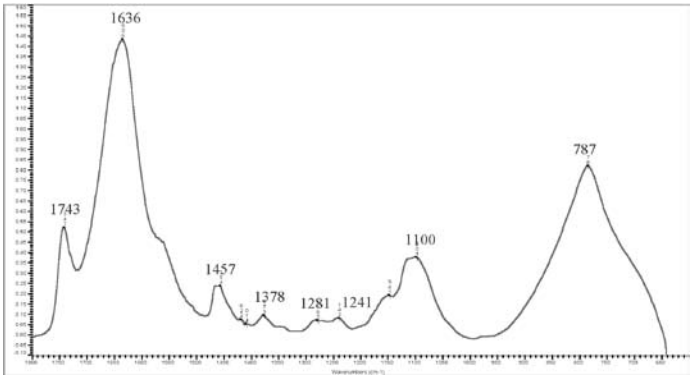
Overview

A comprehensive, multi-instrument, Raman spectroscopic study of wet blue bovine leather was performed, as an extension of our previous infrared absorption studies of in-process bovine hides[1]. Absorption in the mid and far infrared (2000-50 cm^{-1}) showed typical protein features and some environmental contributions, largely from salts[2]. These results were obtained from both attenuated total reflection and diffuse reflection sampling of raw, pickled and in-process wet blue hides. Unfortunately, very little chromium-based vibrations were seen. Raman spectroscopy has many significant features and benefits as a vibrational spectroscopy technique[3]. Raman spectroscopy is completely complementary to infrared absorption spectroscopy. Raman is highly selective for symmetric, polarizable functional groups. Further, Raman is ideal for materials characterization in that there is excellent sampling versatility. Finally, Raman spectroscopy is easily-adapted for in-situ process analysis. All these features were apparent in the citation of Frushour and Koenig's work on the infrared spectroscopy of collagen[4] by Bienkiewicz[5]. We, therefore, hoped that the low frequency Raman scattering spectrum of wet blue leather would show, with high quality, the specific organo-chromium information of this unique sample type. And, consequently, we could directly observe the chromium-collagen complex for the first time.

Dispersive Versus Fourier Transform Raman Spectrometers

At the outset, one might ponder the issues of multi-instruments and different optical approaches to the modern Raman scattering measurement. We decided on a multi-instrument study so that the absolute and relative merits of different instrument types and manufacturers could be properly factored, as if prior to a major instrument purchase. Which instrument would produce the best analytical performance, in other words. We also wanted to compare the different optical platforms currently available in these instruments, namely dispersive and Fourier Transform designs. Typically, a high resolution monochrometer is coupled to a visible or near infrared laser for excitation and the Raman scattering signal is mechanically scanned[3]. Intensity is measured as a function of Raman shift, in wavenumbers (cm^{-1}), from the Rayleigh line of the excitation laser. Spectral acquisition time depends on the optical signal strength and the scan rate of the spectrometer. Usually, 5 to 10 minutes are required to generate a Raman scattering spectrum from a dispersive instrument. A Fourier Transform Raman spectrometer[6], generally employs a Michelson interferometer, Raman notch filter and spatial filter. In this design, all components of the Raman scattering spectrum are present simultaneously at the detector. As the moving mirror of the Michelson scans it presents these spectral signals in and out of phase. The Raman scattering spectrum, Intensity as a function of Raman shift, is constructed by applying a Fourier Transform algorithm to the composite signal. This entire process takes less than a minute. In addition, the Fourier Transform approach offers signal averaging by repeated scanning of the spectrum and high optical throughput, appropriate for weak spectral signals and features. A relatively new type of dispersive Raman spectrometer is based on holographic transmission grating and an array detector[7]. The high optical throughput and multichannel advantage of the Fourier Transform design are contained in what has come to be termed "holographic spectrometer" design.

A: Attenuated Total Reflection Finger Print Spectrum



B: Diffuse Reflectance Fingerprint Spectrum

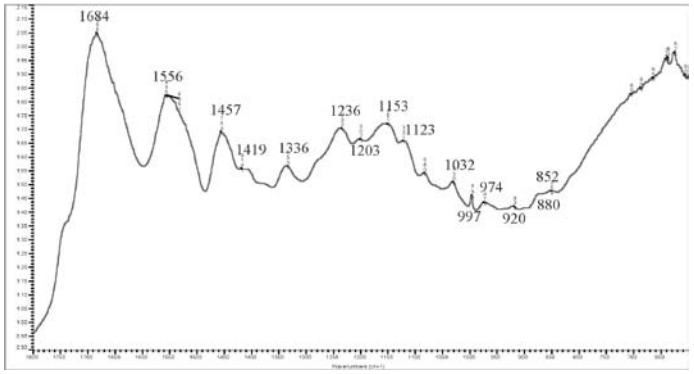


Figure 1: Infrared Absorption Spectra of Wet Blue Bovine Leather

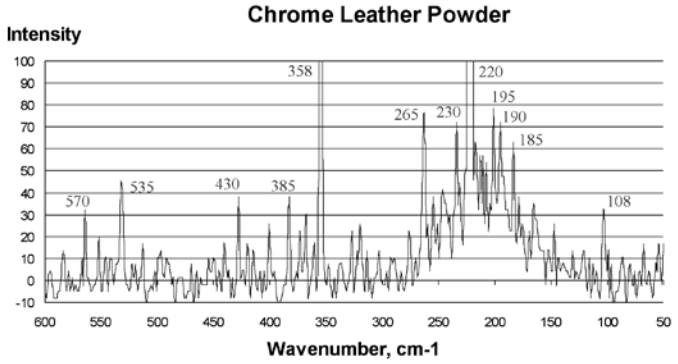


Figure 2: Classic Raman Spectroscopy of Wet Blue Leather Powder; Jarrel-Ash 250 Raman Spectrometer

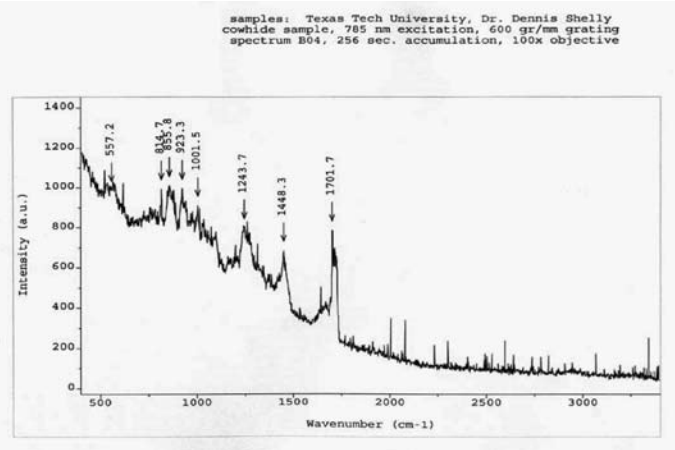


Figure 3: Modern Dispersive Raman Full Scan Spectrum of Wet Blue Leather Slice; Spex-JY Raman 500 Spectrometer

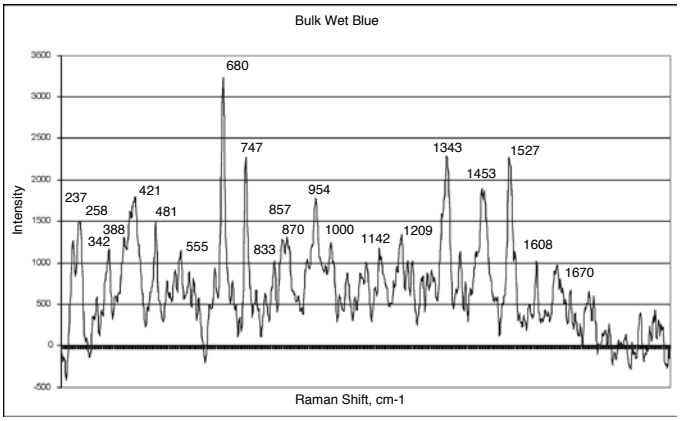


Figure 4: Dispersive Raman Spectrum of Wet Blue Leather Slice; DeltaNu ExamineR 785

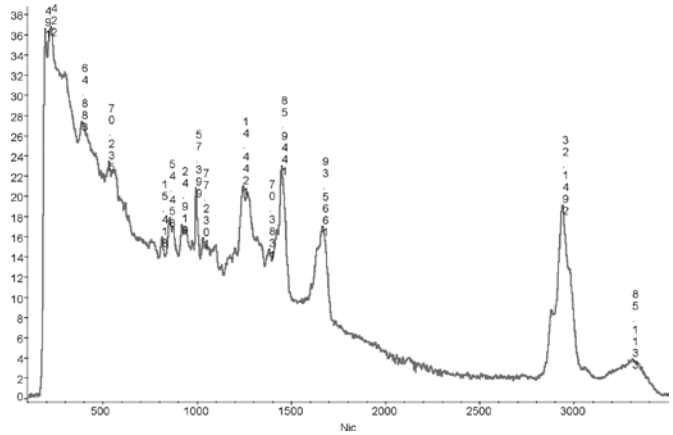


Figure 5: FT Raman Full Scan Spectrum of Wet Blue Leather Slice; Nicolet 960 Spectrometer

Excitation Sources and Sampling

Raman scattering is inelastic scattering of exciting light due to polarizability of a given chemical bond. It is a type of vibrational spectroscopy and many times the Raman scattering spectrum mirrors the infrared absorption spectrum. One can use visible and near infrared discrete spectral sources for the Raman scattering experiment. As such, Argon ion, Helium neon, diode and near infrared solid state lasers can be used, depending on sample absorption characteristics. In general, the closer one comes to electronic excitation with the exciting laser the greater the Raman scattering signal. This is the resonance Raman scattering phenomenon. The Raman scattering signal, in general, is proportional to the inverse fourth power of the exciting light wavelength[4]. However, one must be cognizant of sample fluorescence, which can overpower the Raman scattering signal in the spectrometer and detection system.

Applicable sampling geometries can be found in references 5-7. The simplest and most effective approach is to collect the Raman scattering over a large solid angle (typically at 90° with respect to excitation) and focus that optical signal onto the spectrometer optics, be they dispersive or Fourier Transform. Again, one needs to be aware of photobleaching, photoablation and other deleterious effects of high powered excitation sources on the sample. Careful monitoring of sample degradation will prevent distortion and non-linear effects.

The present Raman scattering spectroscopic study was performed in order to access the low frequency, very weak organo-chromium vibrations, expected in the 900-100 cm^{-1} region[8] for wet blue bovine leather. Indeed, such peaks were observed in the present work. This paper is an overview of the instrumental and sampling requirements leading to these observations. Observed peaks and spectral features are presented. Implications for protein microstructure and process analysis applications are presented.

EXPERIMENTAL SECTION

Samples

Wet blue bovine leather was sampled as thick razor-blade cut slices or as a powder. Wet blue powder was prepared by milling with a Fritsch High Speed Knife Mill # 19 (Gilson Co.Lewis Center, OH). In all cases the leather had dried to ambient humidity, approximately 25%.

FTIR Absorption Analysis

A Thermo-Nicolet Magna 550 FTIR (Madison, WI) was used to acquire baseline infrared absorption spectra of the wet blue leather. A Gemini (Thermo-Spectra Tech, Waltham, MA) attenuated total reflection (ATR)/diffuse reflectance (DRIFTS) accessory was used for an intact hide section (ATR) and for leather powder (DRIFTS), respectively. In the case of DRIFTS, the leather powder was mixed with KBr powder, 10 parts KBr to 1 part leather powder.

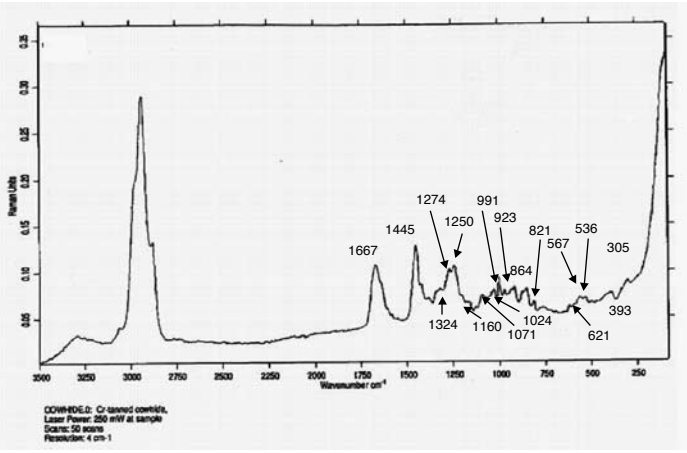
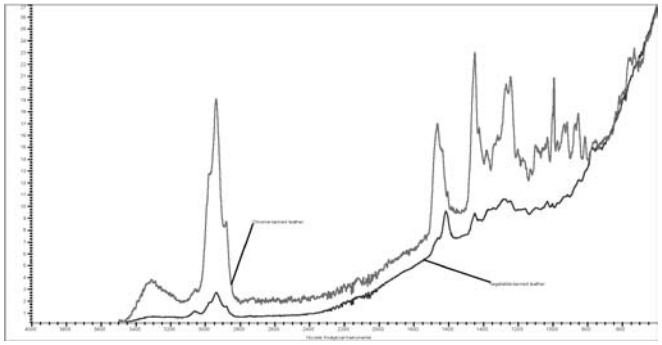


Figure 6: FT Raman Full Scan Spectrum of Wet Blue Leather; Bruker FRA100FT Spectrometer

A: Full Scan Spectra; Red (top): Wet Blue Leather; Blue (bottom): Veg-tanned Leather



B: Fingerprint Spectra; Red (top): Wet Blue Leather: Blue (bottom): Veg-tanned Leather

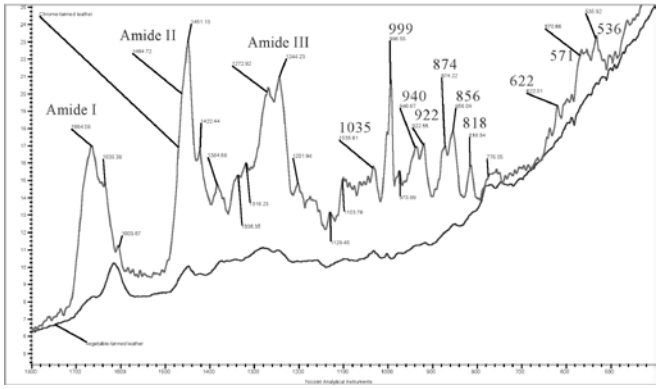


Figure 7: FT Raman Spectra of Wet Blue Leather; Perkin-Elmer 2000R

Dispersive Raman Spectrometers

Three different dispersive Raman spectrometers were used. A Jarrel-Ash 250 Raman (Jarrel-Ash, Anaheim, CA) instrument was available in the Department of Chemistry and Biochemistry at Texas Tech. This instrument utilized a 350 mWatt Argon ion laser at 488 nm, a high resolution (4 cm^{-1}) monochrometer and a computer controlled photon counting system for data acquisition. A Spex – JY Raman 500 instrument (Spex-JY, Metuchen, NJ) was set up for our samples, by the manufacturer in their application lab. This instrument used a 100 mWatt 785 nm diode laser coupled to a R1000 polychrometer and dioide array detector. Finally, DeltaNu Corporation (Laramie, WY) provided time on their ExamineR 785 instrument. A razor cut wet blue leather slice was examined through a 10x objective in this microscope-based instrument which employed a 785 nm diode laser. These instruments follow the general theme of a classic Raman spectrometer [3,6].

FT Raman Spectrometers

Three different Fourier Transform (FT) Raman instruments were used. Thermo-Nicolet (Madison, WI) contributed with a Raman 960 instrument. Bruker Instruments (Billerica, MA) donated time on their FRA 100FT instrument. And, Perkin-Elmer (Hartford, CT) provided a 2000R FT instrument. All three of these instruments used Nd-YAG lasers providing 1064 nm near infrared light for Raman excitation. All three instruments followed the basic there of a FT Raman instrument design[6].

Holographic Grating Spectrometer

Kaiser Optics Inc. (Ann Arbor, MI) donated time on their HoloProbe instrument. A system diagram for this instrument can be found in reference 7. Key components are the holographic transmission grating, large numerical aperture focusing optics, interference filters, holographic notch filter and diode array detector. This design has high optical throughput and rapid spectral acquisition.

RESULTS AND DISCUSSION

Baseline FTIR Spectra

Figures 1 A and B show infrared absorption spectra for wet blue leather in the fingerprint region (1800 to 600 cm^{-1}). Figure 1A is an ATR spectrum while Figure 1B is a DRIFTS spectrum. In both spectra the absorption intensity is very high, but, (especially Figure 1B) the bandwidth is low, i.e. there is inherently low spectral resolution. Neither spectra were corrected for reflectance artifacts. This was somewhat surprising and disappointing. However, we did not exhaust all our options in improving these FTIR results, choosing instead to put our efforts into the Raman spectroscopy. Nonetheless, the spectra of Figure 1 show us the infrared-active bands in the fingerprint region.

Dispersive Raman Spectra

Our first Raman investigation was based on the Jarrel-Ash Raman 250 instrument, described above. Figure 2 is a Raman scattering spectrum of wet blue leather powder from this instrument. These data were imported into Microsoft Excel™ and displayed as an X-Y graph. This spectrum appears to be characterized by noise and rather poor Raman signal strength, in general. The very large sharp peaks in both spectra might

be due to off-resonance, satellite, laser emission lines. As such, they could have been minimized with an interference filter in the excitation optical train or a Raman notch filter in the scattering optical train. None of these were available to us, however.

The Spex-JY Raman 500 instrument produced a somewhat better result, as shown in Figure 3. This is a full scan spectrum, from 500 to 3500 cm^{-1}. There are eight marked peaks on a sloping baseline. This sloping baseline is worse at low Raman shift (i.e. closer to the Rayleigh line), which suggests sample fluorescence interference. Overall, the signal to noise ratio is rather poor, hardly what one would characterize as a high quality spectral result. We, therefore, specified to the various instrument vendors that sample fluorescence was a potential serious interference with 785 nm excitation and proper protections/corrections should be enacted. One possibility was to use 1064 nm exciting lasers be used for evaluations.

Finally in this category, we observe the DeltaNu ExamineR 785 instrument's spectrum in Figure 4. As in Figure 2, these data were imported into Microsoft Excel™ and plotted as an X-Y graph. We see striking similarity between Figure 2 and Figure 4, here. Thus, we now consider Figure 2 as genuinely valid data. There are 21 peaks in Figure 4, indicating good signal to noise performance and sensitivity for the spectrometer system. It is quite likely that imaging with a 10x microscope objective helps in this regard. Another feature of this instrument was baseline compensation, a feature that helped in the presentation of these data.

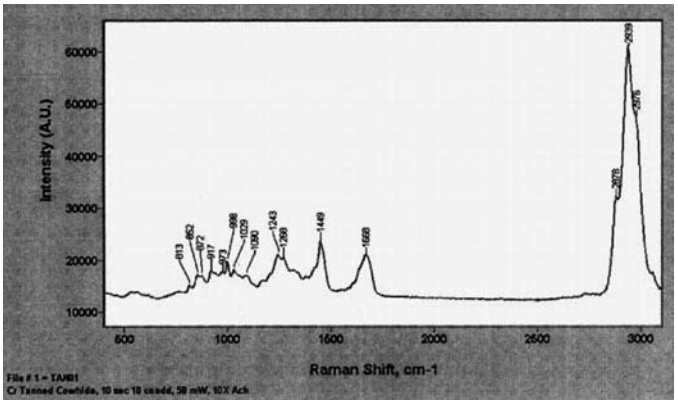
FT Raman Spectra

A Thermo-Nicolet 960 FT instrument provided the spectrum of Figure 5. This is a very good full scan spectrum, clearly showing protein regions, as well as several peaks below 800 cm^{-1}. The sloping background does detract from data quality, however. The signal to noise ratio is only moderate, however, especially evident in the 2500 to 2000 cm^{-1} region.

A Bruker FRA100FT instrument provided the full scan spectrum shown in Figure 6. The signal to noise ratio, overall, is much better than that for the dispersive instruments and appears to be somewhat better than that of the Nicolet 960 FT instrument.

A Perkin-Elmer 2000R FT instrument was used to generate the spectra shown in Figure 7. Figure 7A is a full scan spectrum and Figure 7B is a scan in the fingerprint region. This specially-tuned instrument (see instrument configuration above) produced the best results of the FT instruments. There is excellent signal to noise, even on a sloping baseline below 800 cm^{-1}.

A: Full Scan Spectrum



B: Fingerprint Spectrum

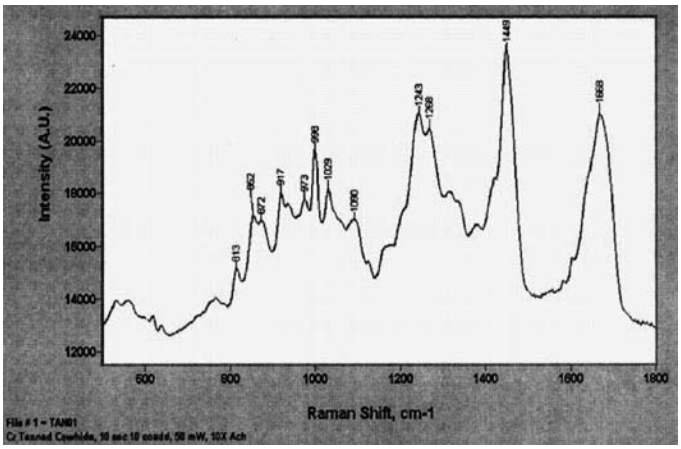


Figure 8: Modern Holographic Raman Spectra of Wet Blue Leather; Kaiser Optical Holoprobe

Holographic Raman Spectra

A Kaiser Optical HoloProbe instrument was used to yield the spectra of Figure 8. Figure 8A is a full scan spectrum while Figure 8B is the fingerprint spectrum. There are 23 peaks recognized in the fingerprint spectrum (Fig. 8B) and the signal to noise performance is excellent. Also, the baseline has been compensated without compromise on low frequency band strength.

Summary of Raman Spectral Results

Band assignments for selected spectral features present in the fingerprint spectra are shown in Table I. Note the typical protein bands and those features due to HSO_4^- and SO_4^{2-}. Note, also, those bands attributable to chromium[8-12]. Of particular interest is the Cr-O-Cr bend at 855 cm^{-1}, the octahedral Cr-X stretch around 550 cm^{-1} and "unspecified" Cr stretch between 300 and 500 cm^{-1}. The presence (or absence) of these specific features for the seven instruments is noted in Table II. Table II lists 35 bands in the mid to far IR that were found in the Raman scattering spectra (infrared absorption data not shown in this table). In general there is good correspondence among the seven instruments. However,

TABLE I
Band Assignments for Wet Blue Leather[4,8-12]

Band/Peak (cm^{-1})	Assignment
1669	Amide I Complex, C=O stretch
1450	Amide II Complex, N-H bend + C-N stretch
1340-1206	Amide III Complex, C-O stretch
1168	asym S-O stretch in HSO_4^-
1096	S=O stretch in SO_4^{2-}
1031	sym S-O stretch in HSO_4^-; monomeric Cr=O stretch
994	unassigned
920	Cr-O stretch in CrO_4^{2-}; C-C stretch of protein backbone
873	Cr-O-Cr bend (880)
855	HSO_4^- [S-O(H) stretch]; Cr-O-Cr bend; C-C stretch of residues
813	Cr-O bend in CrO_4^{2-}; C-C stretch of Hyp ring
820-700	NH_2 wagging in amines; C-C stretch of Pro ring
700-600	S-O bending in SO_4^{2-}
570-520	octahedral Cr 3^+ (550)
500-300	unspecified Cr
300-100	unassigned

each one has certain advantages and limitations. The Jarrel-Ash dispersive instrument did not cover the fingerprint mid-IR region at all, whereas it gave good peaks in the far-IR region (see Figure 2). The Perkin-Elmer FT instrument did very well in the fingerprint region, 1800 to about 600 cm^{-1}. Most of the bands that the Jarrel-Ash dispersive instrument found are unassigned at this time, unfortunately. We have not found assignments in the open literature[11-14] and we have not performed *ab initio* calculations to attempt assignments at this time.

The columns in Table II reflect instrument-specific responses while the rows suggest measurement-specific responses. Both the DeltaNu dispersive and the Perkin-Elmer FT instruments recorded 23 hits in their respective columns in the table. Third was the Kaiser Optics holographic spectrometer with 17 hits in its column. The Thermo-Nicolet 960 and the Bruker FRA900 FT instruments scored 13 matches in their respective columns. The Jarrel-Ash 250 scored 12 hits and the Spex-JY dispersive instrument scored 8 peaks in its column in the table. Further, there were 10 rows (frequencies) that recorded scores of five and higher, indicating that these ten bands are truly significant spectral features and not random perturbations. These are 1450, 1340-1206, 1031, 994, 920, 855, 813, 560, 540 and 390 wavenumbers. The underlined might be attributable to chromium. As shown in Table I, the peak at 994 cm^{-1} is unassigned at this time. Also, comparing Figure 2 with Table II, there are seven additional bands that are unassigned at this time. If many can be attributed to Cr-R vibrations (where R = any substituent), one could say that the Raman spectrum from the Amide III complex all the way to the Rayleigh line was dominated with chromium-organic resonances, making this a truly rich spectral environment.

Another common feature of most of the Raman spectra is the relatively high band strength in the mid-to-low frequencies. This indicates that the attributed vibration is strong, i.e. the bond is polarizable generating good Raman signals. Such would likely be the case for Cr-R bonds, as we had hoped from the outset. We feel that Raman is the preferred technique for Cr-R –containing functional groups and we shall continue to pursue Raman for chrome tanned leather analyses in the future.

CONCLUSIONS

Raman spectroscopy is an excellent instrumental tool to study chrome tanned leather, giving much important spectral data for this material. Thirty five peaks were recorded, as a composite of the seven instruments used in this study. This actually represents quite a bit of good spectral data from which to do protein materials characterization; which is exactly what we hoped for.

Raman spectroscopy is complementary to infrared absorption spectroscopy but offers unique advantages in actual practice, particularly with respect to leather materials analysis. We not only saw good correspondence between infrared absorption and Raman scattering, but much more versatility in Raman scattering, as evidenced by the increased spectral coverage without changing sampling configurations. The ATR sampling technique of FTIR could not extend below 600 cm^{-1}, for example, due to significant absorption by the crystal material (ZnSe).

FT instrumentation uniformly produced excellent results, compared to classic dispersive Raman configuration. Results from the Perkin-Elmer 2000R and the DeltaNu dispersive instrument were nearly identical and led the instruments in spectral quality and utility. The Kaiser Optical Holographic instrument was clearly second, as regards number of spectral

TABLE II

Vibrational Spectroscopy Results Summary and Comparison

Band/Peak	Jarrel-Ash Dispersive	Spex-JY Dispersive	DeltaNu Dispersive	Thermo-Nicolet FT	Bruker FT	Perkin-Elmer FT	Kaiser Optics Holographic
1690		X	X			X	
1669			X	X	X		X
1530			X			X	
1450		X	X	X	X		X
1410						X	
1380				X		X	X
1340-1206		X	X	X	X	X	X
1168					X	X	X
1130						X	
1096			X			X	X
1031			X	X	X	X	X
994		X	X	X	X	X	X
941			X			X	
920		X	X	X	X	X	X
873			X			X	X
855		X	X	X	X	X	X
813		X		X	X	X	X
762	X					X	X
747			X				
680			X				
645			X			X	X
620					X	X	X
560	X	X	X		X	X	X
540	X		X	X	X	X	X
481			X				
430	X		X				
390	X		X	X	X	X	
350	X		X			X	
265	X		X				
230	X		X				
220	X			X			
195	X			X			
190	X						
185	X						
145						X	

peaks displayed and overall spectral quality. The Jarrel-Ash 250 Raman dispersive instrument performed almost as well as did the Thermo-Nicolet 960 and the Bruker FRA100 FT instruments. The Spex-JY dispersive instrument was somewhat inferior to the remaining six spectrometers, in terms of spectral quality. Yet, all seven instruments generated some data at nearly identical frequencies, suggesting that the fundamental Raman phenomenon could be recorded by either instrument configuration with good accuracy and precision.

A companion benefit to Raman instrumentation, in general, is the efficient sampling of materials. This especially is important to in-process analysis. It should be possible, therefore, to study chrome complexation with Raman as a vastly superior technique to infrared absorption. Though this was not part of the present study, the literature shows that in-process materials characterization is one of the key spectroscopic applications for Raman scattering. For example, the new class of dispersive spectrometers, holographic spectrometers, are uniquely adaptable for process analysis[5]. This is one of our objectives for the future. Presumably, one would be able to differentiate between bound and free chrome and between "new" and "old" (or aged) wet blue leather using Raman scattering as a probative tool.

We hope to be able to report completed band assignments in the Raman spectrum of wet blue leather in the near future. This is a significant task but one that really needs to be done if we are to fully understand chrome tanned leather and all the nuances presented in the chemistry of this unique collection of protein materials. It is worth mentioning that the chromium-collagen complex(es) have yet to be directly observed by experimental methods, despite more than 100 years of industrial practice[15]. We trust that Raman spectroscopy is just the tool required for this important measurement.

ACKNOWLEDGEMENTS

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